The Journal of Experimental Biology 211, 35-41
Published by The Company of Biologists 2008
doi:10.1242/jeb.012658

Development partly determines the aerobic performance of adult deer mice, *Peromyscus maniculatus*

Gregory A. Russell[1,*], Enrico L. Rezende[2] and Kimberly A. Hammond[1]

[1]Department of Biology, University of California at Riverside, Riverside, CA 92521, USA and [2]Integrative Ecology Group, Estación Biológica de Doñana, CSIC, Apdo. 1056, E-41080 Sevilla, Spain

*Author for correspondence (e-mail: gruss001@student.ucr.edu)

Accepted 24 October 2007

SUMMARY

Previous studies suggest that genetic factors and acclimation can account for differences in aerobic performance ($\dot{V}_{O_2max}$) between high and low altitude populations of small mammals. However, it remains unclear to what extent development at different oxygen partial pressures (P_{O_2}) can affect aerobic performance during adulthood. Here we compared the effects of development at contrasting altitudes *versus* effects of acclimation during adulthood on $\dot{V}_{O_2max}$. Two groups of deer mice were born and raised for 5 weeks at one of two altitudes (340 and 3800 m above sea level). Then, a subset of each group was acclimated to the opposite altitude for 8 weeks. We measured $\dot{V}_{O_2max}$ for each individual in hypoxia (P_{O_2}=13.5 kPa, 14% O_2 at 3800 m) and normoxia (P_{O_2}=20.4 kPa, 21% O_2 at 340 m) to control for P_{O_2} effects. At 5 weeks of age, high altitude born mice attained significantly higher $\dot{V}_{O_2max}$ than low altitude born mice (37.1% higher in hypoxia and 72.1% higher in normoxia). Subsequently, deer mice acclimated for 8 weeks to high altitude had significantly higher $\dot{V}_{O_2max}$ regardless of their birth site (21.0% and 72.9% difference in hypoxia and normoxia, respectively). A significant development × acclimation site interaction comparing $\dot{V}_{O_2max}$ in hypoxia and normoxia at 13 weeks of age suggests that acclimation effects depend on development altitude. Thus, reversible plasticity during adulthood cannot fully compensate for developmental effects on aerobic performance. We also found that differences in aerobic performance in previous studies may have been underestimated if animals from contrasting altitudes were measured at different P_{O_2}.

Key words: acclimation, aerobic performance, hypoxia, developmental canalization, phenotypic plasticity, $\dot{V}_{O_2max}$.

INTRODUCTION

Physiological ecologists have always been interested in how organisms accommodate extreme environmental conditions such as cold (Almeida-Val et al., 1994; Guderley and St-Pierre, 1996), high salinity (Tay and Garside, 1975) and hypoxia (Hochachka, 1988; Singer, 1999; West, 1991). Recently, much attention has focused on how humans and other mammals cope with the rigors of montane and other high altitude environments (e.g. Hochachka et al., 1982; Curran et al., 1998; Hammond et al., 1999; Hammond et al., 2001; Hammond et al., 2004; Hayes, 1989a; Hayes, 1989b; Hayes and Shonkwiler, 1996; Hayes and O'Connor, 1999; Rezende et al., 2001; Rezende et al., 2005; Sheafor, 2003). In particular, small mammals living at high altitude face several important challenges because of their size. Energy demands increase because they are active in low ambient temperatures, but hypoxia limits aerobic metabolism so it is harder to fuel these needs. Additionally, primary productivity is often low at high altitude so there are fewer resources to fuel higher demands (Hammond et al., 2004).

Studies comparing populations from high and low altitudes have reported a variety of physiological differences that were initially interpreted as adaptations (in a Darwinian sense) to different altitudes and oxygen partial pressures (P_{O_2}). However, subsequent studies have shown that chronic exposure to high altitude (i.e. acclimation or acclimatization) can result in important physiological responses (e.g. McClelland et al., 1998; McClelland et al., 2001), suggesting that differences between populations might be partly determined by phenotypic plasticity. Although some studies have attempted to control for acclimatory effects when comparing aerobic performance across populations or species inhabiting different altitudes (e.g. Rezende et al., 2001; Hammond et al., 2001), the role of development as a source of variation has not been previously addressed (but see Chappell et al., 2007). Disturbances of the developmental process, whether genetic, environmental or phylogenetic, may not be reversible (developmental canalization) and can result in significant variability within a species (Spicer and Gaston, 1999; Dzialowski et al., 2002; Spicer and Burggren, 2003).

Therefore, we tested whether developmental effects can contribute to variation in aerobic metabolism during adulthood. We focused on maximum aerobic performance during exercise ($\dot{V}_{O_2max}$) because of the large role aerobic exercise plays in an organism's everyday life, particularly at high altitude (Pough, 1980; Hayes and Shonkwiler, 1996). We used the North American deer mouse (*Peromyscus maniculatus* Le Conte) as a model for study for several reasons. Deer mice inhabit a wide altitudinal range, from below sea level to above 4000 m (Hock, 1961) and are North America's most widespread mammal. They also display an array of polymorphisms in the α-chains of their hemoglobin that are geographically correlated with altitude (Snyder, 1981; Snyder et al., 1988), influence blood oxygen affinity, and differentially affect aerobic metabolism at low and high altitude (Chappell and Snyder, 1984; Chappell et al., 1988). Finally, field studies at high altitude suggest that natural selection favors high

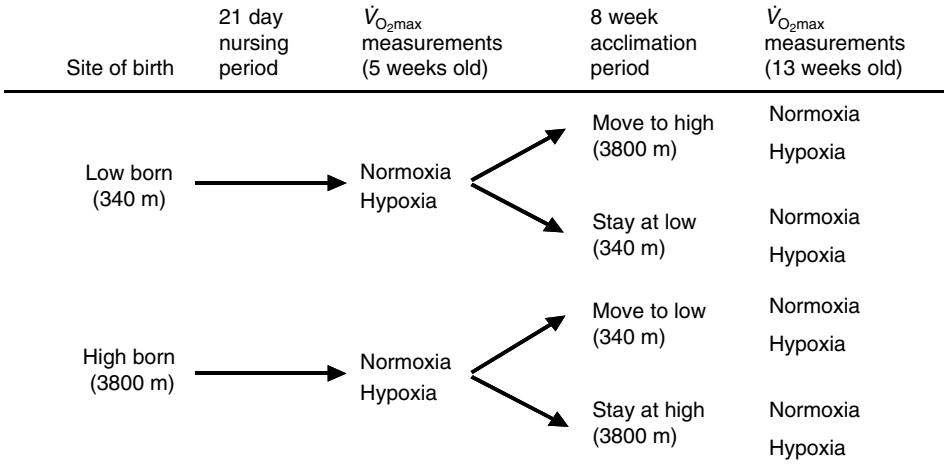

Fig. 1. Experimental design employed in this study. Testing conditions are described within each column, sample sizes and results are summarized in Table 1.

aerobic capacity during thermogenesis in *P. maniculatus* (Hayes and O'Connor, 1999), hence our results are certainly relevant in an evolutionary context.

We quantified whether effects of development at high altitude exist and to what extent variation in aerobic performance might be associated with developmental canalization. With this purpose, we estimated and compared effects associated with two sources of phenotypic plasticity: developmental plasticity (during *in utero* development and growth) and reversible plasticity during adulthood. If developmental canalization and physiological heterokairy [changes in the timing and/or onset of a particular physiological system during development (Blacker et al., 2004)] are partly responsible for variation in aerobic performance during adulthood, we expect that reversible acclimation cannot fully compensate for the effects of developmental acclimation.

MATERIALS AND METHODS

Experimental animals and design

For this study we used individuals of *P. m. sonoriensis* from a colony that is 4–7 generations removed from the wild (from a founder population trapped near Mt Barcroft, about 3800 m elevation, in eastern California). The breeding program in the colony was managed to maximize outcrossing and there was no intentional selection. To obtain two groups of mice born at different altitudes (high *versus* low), breeding pairs were established at the Barcroft Laboratory of the University of California's White Mountain Research Station (elevation 3800 m; 14% O_2 or P_{O_2}=13.5 kPa) and at Riverside (elevation 340 m; 21% O_2 or P_{O_2}=20.4 kPa). From these breeding pairs, we obtained 20 offspring born at low altitude and 19 born at high altitude. At 5 weeks of age, half of the offspring of each group were moved and acclimated to the opposite altitude for an additional 8 weeks. Transportation between Riverside and Barcroft took approximately 6–7 h in an air-conditioned vehicle; the majority of the ride was over smooth highway and care was taken not to cause undue stress to the animals during this time. Therefore, we ultimately had four treatment groups (Fig. 1; Table 1): (1) low born/low measured; (2) low born/high measured; (3) high born/high measured; and (4) high born/low measured.

All mice were housed individually in plastic shoebox cages (27 cm × 21 cm × 14 cm) at 23–25°C. They were given *ad lib* food (23% protein, 4.5% fat, 6% fiber, 8% ash and 2.5% minerals), water and bedding. In the lab, they were maintained on a photoperiod cycle that approximates the natural cycle at Barcroft during the summer months (i.e. ~14 h:10 h L:D in mid-July).

$\dot{V}_{O_2max}$ during exercise

Maximum $\dot{V}_{O_2}$ was determined using open flow respirometry by running mice in an enclosed motorized treadmill. The treadmill's working section (the portion of the total enclosed gas volume that the mouse was constrained to) was 6 cm wide, 7 cm high and 13 cm long, and the enclosed total gas volume was approximately 970 ml. We used a flow rate of approximately 2100 ml min^{-1}, standard temperature and pressure (STP) of dry air. Gas flow was regulated with Tylan and Applied Materials mass flow controllers (Santa Clarita, CA, USA) upstream from the treadmill. Approximately 100 ml min^{-1} of excurrent gas was scrubbed of CO_2 and water vapor (using soda lime and drierite, respectively) and routed through the oxygen sensors. Changes in O_2 concentration were measured with Ametek/Applied Electrochemistry S-3A analyzers (Naperville, IL, USA) and recorded on a Macintosh computer with a National Instruments A–D converter (Austin, TX, USA)

Table 1. Sample size, body mass and aerobic performance means (± s.e.m.) for each of the two groups at 5 weeks of age and the four groups at 13 weeks of age

	5 weeks of age					13 weeks of age			
Birth site	*N*	Body mass	$\dot{V}_{O_2max}$ hypoxia	$\dot{V}_{O_2max}$ normoxia	Acclimation site	*N*	Body mass	$\dot{V}_{O_2max}$ hypoxia	$\dot{V}_{O_2max}$ normoxia
Low	20	18.03±0.74	3.31±0.11	3.98±0.28	Low	10	22.78±0.86	3.98±0.17	4.05±0.41
					High	10	21.86±0.86	5.32±0.17	8.64±0.40
High	19	17.38±0.50	4.54±0.11	6.85±0.28	Low	6	21.73±1.11	4.31±0.22	4.89±0.52
					High	13	21.86±0.75	4.69±0.16	6.71±0.37

See Fig. 1 for experimental design. Body mass is given in g, and $\dot{V}_{O_2max}$ is given in ml O_2 min^{-1}. Aerobic performance values are body mass corrected.

using custom-made data acquisition software (http://warthog.ucr.edu). We calculated $\dot{V}_{O_2}$ (in ml min^{-1}) as:

$$\dot{V}_{O_2} = \dot{V} \times (F_{IO_2} - F_{EO_2}) / (1 - F_{EO_2}) ,$$

where $\dot{V}$ is the flow rate (ml min^{-1}; STP) and F_{IO_2} and F_{EO_2} are the fractional oxygen concentrations of incurrent and excurrent gases, respectively.

To begin a test, after measuring body mass, we placed a mouse in the treadmill's enclosed chamber and allowed for a 3–5 min adjustment period before starting the tread at low speed (approximately 0.1 m s^{-1}). We continued to increase speed in increments of 0.1 m s^{-1} every 30 s until the mouse could either no longer maintain position on the tread or $\dot{V}_{O_2}$ did not increase with increasing speed, at which time the tread was stopped. At the end of the exercise we confirmed that $\dot{V}_{O_2}$ fell rapidly; all mice showed signs of exhaustion but none were injured. Tests lasted a total of 6–20 min. Reference readings of incurrent gas were obtained at the beginning and end of the trial.

Due to the treadmill's relatively large volume, we applied the 'instantaneous' correction (Bartholomew et al., 1981) to compensate for mixing characteristics and to resolve short-term changes in metabolic rate. The effective volume of the treadmill respirometry chamber, calculated from washout curves, was 903 ml. We computed $\dot{V}_{O_2max}$ as the highest instantaneous $\dot{V}_{O_2}$ averaged over continuous 1 min intervals (Chappell et al., 1995).

Measurements of aerobic performance for each individual were carried out at the end of the developmental period (5 weeks of age) and after acclimation (13 weeks of age), at two different P_{O_2} to obtain comparable measurements simulating high and low altitudes. In Riverside, measurements were performed with ambient air (normoxia, P_{O_2}=20.4 kPa) and employing a gas mixture of 14% O_2 and 86% N_2 (hypoxia, P_{O_2}=13.5 kPa). Similar P_{O_2} values were obtained in Barcroft employing ambient air (hypoxia) and a mixture of 32% O_2 and 68% N_2 (normoxia). Despite the mix appearing hyperoxic, these testing conditions approximate the 'normoxic' conditions encountered in Riverside. Ambient P_{O_2} in Riverside (340 m above sea level) is ~20.4 kPa, but at Barcroft (P_{O_2} ~13.5 kPa), a P_{O_2} of 20.4 kPa can only be achieved by exposing the animal to a fractional O_2 content of 0.32; in this instance, barometric pressure must be taken into account to know the true amount of oxygen available to the animal. From here on, we will refer to measurements made at 20.4 kPa as normoxic and measurements made at 13.5 kPa as hypoxic.

Analyses and statistics

We initially assessed how body mass and aerobic performance of our mice changed with age, controlling for effects of developmental and acclimatory altitudes (below). This was carried out using repeated-measures ANOVA comparing body mass and aerobic performance obtained at 5 weeks *versus* 13 weeks of age. Because aerobic performance was measured at two different P_{O_2}, we performed separate repeated-measures ANOVAs for hypoxia and normoxia. In addition, we performed pairwise Pearson correlations between residuals controlling for development and acclimation site (and for mass differences in the case of $\dot{V}_{O_2max}$) to determine whether body mass and aerobic performance were repeatable across ages and different P_{O_2}.

Subsequently, several analyses were performed to disentangle the effects of development and acclimation. To estimate developmental effects in aerobic performance at 5 weeks of age, we compared the aerobic performance of mice born at high *versus* low altitude with an analysis of covariance (ANCOVA), with birth altitude as the main effect and body mass as a covariate. Because mice were tested twice at different P_{O_2}, separate ANCOVAs were performed for measurements in hypoxia and normoxia. To determine whether responses to different P_{O_2} varied as a function of birth site, we employed a repeated-measures ANOVA comparing the aerobic performance of each individual in hypoxia *versus* normoxia, with birth site as a between-subject factor.

We then compared aerobic performance obtained at 13 weeks of age (5 weeks of development followed by 8 weeks of acclimation) for the same individuals, to partition the effects of development *versus* acclimation in $\dot{V}_{O_2max}$. We employed an ANCOVA with both birth altitude and acclimation altitude as main effects and body mass as a covariate (analyses were performed separately for hypoxia and normoxia). To determine whether individuals within groups showed different responses to P_{O_2} during aerobic performance measurements as a function of development and/or acclimation, we performed a second repeated-measures ANOVA with birth site and acclimation site as between-subject factors. Unless stated otherwise, F values are from these statistical tests and we used an alpha value of 0.05 for statistical significance.

Table 2. Repeatability of aerobic performance and body mass across different P_{O_2} or 8 weeks of acclimation

	Raw values		Mass-corrected values	
	r	*P*	*r*	*P*
P_{O_2} – hypoxia *versus* normoxia				
$\dot{V}_{O_2max}$ at 5 weeks	0.66	**<0.0001**	0.49	**0.002**
$\dot{V}_{O_2max}$ at 13 weeks	0.59	**<0.0001**	0.38	**0.02**
Age – 5 weeks *versus* 13 weeks				
Body mass	0.71	**<0.0001**	–	–
$\dot{V}_{O_2max}$ in hypoxia	0.53	**0.001**	0.20	0.23
$\dot{V}_{O_2max}$ in normoxia	0.18	0.28	–0.14	0.41

Results were estimated as Pearson product-moment correlations between ANOVA residuals controlling only for site of birth and/or acclimation altitude (raw values), or residuals including mass as an additional covariate (mass-corrected values). Statistically significant results according to a one-tailed hypothesis are shown in bold.

RESULTS

Repeatability across P_{O_2} and 8 weeks of acclimation

To test whether a trait is repeatable is to ask (1) whether the individual changed over time and (2) whether that individual maintained its relative rank in the population (with regard to that trait) over time. Aerobic performance measured in normoxia and hypoxia were significantly correlated at both 5 and 13 weeks of age in models controlling for site of birth and/or acclimation altitude, with and without body mass as a covariate (Table 2). After correcting for site of birth and acclimation altitude, body mass was significantly repeatable between 5 and 13 weeks of age (r=0.71, P<0.0001). Raw $\dot{V}_{O_2max}$ measured in hypoxia, but not in normoxia, was significantly correlated after 8 weeks of acclimation (P=0.001 and P=0.28, respectively; Table 2). After accounting for body mass differences, $\dot{V}_{O_2max}$ in hypoxia and normoxia was not repeatable over the 8 weeks of acclimation (Table 2).

Effects of birth altitude and P_{O_2} at 5 weeks of age

In hypoxia (P_{O_2}=13.5 kPa), mice born at high altitude had a 37% higher $\dot{V}_{O_2max}$ that those born at low altitude ($F_{1,36}$=64.1, P<0.0001; Table 1). When tested in normoxia (P_{O_2}=20.4 kPa), mice born at high altitude had a 72% higher $\dot{V}_{O_2max}$ than mice born at low altitude

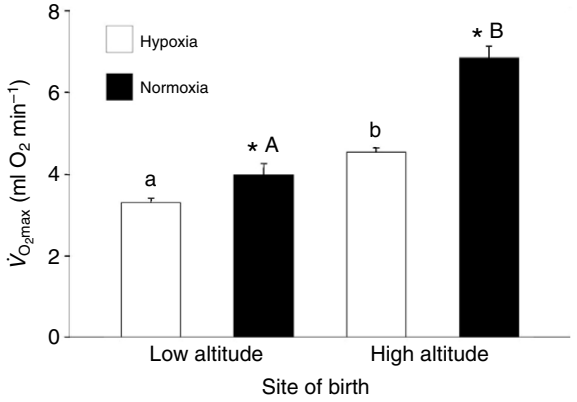

Fig. 2. Aerobic performance at 5 weeks of age measured in hypoxia and normoxia. Asterisks represent statistical significance within birth altitude; lower-case letters represent statistical significance in hypoxia and upper-case letters represent statistical significance in normoxia. Means are body mass-corrected values from ANCOVA and error bars are s.e.m.

($F_{1,36}$=50.7, P<0.0001; Table 1). Accordingly, the repeated-measures ANOVA shows that animals born at high altitude attained a significantly higher $\dot{V}_{O_2max}$ than mice born at low altitude, regardless of P_{O_2} (between-subject effect, $F_{1,37}$=36.87, P<0.0001). Nonetheless, P_{O_2} effects were more pronounced in animals born at high altitude (Fig. 2), which is supported by the significant P_{O_2} × site of birth interaction in this analysis ($F_{1,37}$=70.64, P<0.0001).

Effects of birth *versus* acclimation altitude at 13 weeks of age

Regular ANCOVAs comparing $\dot{V}_{O_2max}$ as a function of birth site and acclimation altitude show that high altitude acclimated mice attained significantly higher $\dot{V}_{O_2max}$ than animals acclimated at low altitude in hypoxia and normoxia ($F_{1,33}$=22.4, P<0.0001 and $F_{1,33}$=56.47, P<0.0001, respectively; Fig. 3). Although the main effects of birth site were not significant in these analyses (P>0.21 in both cases), there was a significant birth altitude × acclimation altitude interaction for $\dot{V}_{O_2max}$ in both hypoxia and normoxia ($F_{1,33}$=6.82, P=0.013 and $F_{1,33}$=10.5, P=0.003, respectively). Among animals acclimated to low altitude, those born at low altitude attained lower $\dot{V}_{O_2max}$ than those born at high altitude, whereas the opposite pattern was observed among mice acclimated to high altitude. In other words, mice acclimated to their 'native' altitude had consistently lower $\dot{V}_{O_2max}$ than those that were switched to the opposite altitude at 5 weeks of age (Fig. 3).

Repeated-measures ANOVAs testing for P_{O_2} effects showed that mice acclimated to high altitude, regardless of birth site, increased aerobic performance by 52% in normoxia compared with measurements in hypoxia (acclimation, $F_{1,34}$=30.44, P<0.0001 and birth site, $F_{1,34}$=1.75, P=0.2). The relative increase in aerobic performance as a function of P_{O_2} was significantly higher in animals acclimated at high altitude (P_{O_2} × acclimation altitude, $F_{1,34}$=25.11, P<0.001; Fig. 3). Separate repeated-measures analyses within each treatment support this conclusion: P_{O_2} effects were significant only in high born/high acclimated and low born/high acclimated groups ($F_{1,11}$=23.27, P=0.001 and $F_{1,9}$=26.61, P=0.001, respectively).

Age effects between 5 and 13 weeks

Body mass increased about 4 g during the 8 week duration of the acclimation (within-individual effect, $F_{1,36}$=166.13, P<0.0001).

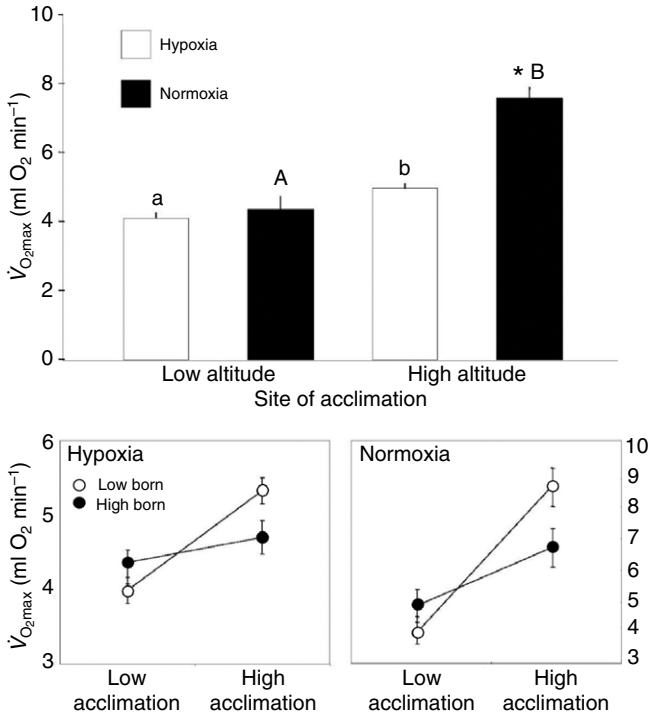

Fig. 3. Top panel, aerobic performance at 13 weeks of age measured in hypoxia and normoxia. Mice were pooled by acclimation altitude because this was the only significant main effect (see Results). Asterisks represent statistical significance within acclimation altitude; lower-case letters represent statistical significance in hypoxia and upper-case letters represent statistical significance in normoxia. Means are body mass-corrected values from ANCOVA and error bars are s.e.m. Bottom panels, adjusted means ± s.e.m. from ANCOVAs performed separately for trials in hypoxia (left) and normoxia (right). The birth site × acclimation altitude interaction was significant in both ANCOVAs (see Results).

Neither birth altitude nor acclimation altitude significantly affected growth rate ($F_{1,36}$=0.34, P=0.56 and $F_{1,36}$=0.32, P=0.58, respectively), and there were no significant interactions (P>0.45 in all cases; Fig. 4).

As for aerobic performance, $\dot{V}_{O_2max}$ measured in hypoxia changed significantly during the 8 weeks of acclimation (within-individual effect, $F_{1,36}$=28.2, P<0.0001; Table 1). Acclimation altitude was a significant between-individual effect, with $\dot{V}_{O_2max}$ being significantly higher in mice acclimated at high altitude regardless of their birth site ($F_{1,36}$=4.84, P=0.03). Nonetheless, all interactions were statistically significant (P<0.005 for age × birth altitude, age × acclimation altitude, and age × birth × acclimation altitude), showing that the magnitude of change during the 8 weeks of acclimation depends on birth altitude, acclimation altitude and the interaction between both (Fig. 4). Results were qualitatively identical for measurements in normoxia.

DISCUSSION

We found no evidence that aerobic performance is repeatable between 5 and 13 weeks of age, after controlling for body mass and birth and acclimation altitude (Table 2). This finding is in contrast to reports of high aerobic performance repeatability in deer mice (Hayes and Chappell, 1990) over the course of 3 months. Chappell et al. (Chappell et al., 1995) reported high exercise aerobic performance repeatability in Belding's ground squirrels (*Spermophilus beldingi*) over short periods (2 h) and over months

or years in adult animals, but noted no repeatability when animals were tested as juveniles and later as adults. This measurement period was significantly longer than ours (1–2 years *versus* 8 weeks), but we do point out that physiological traits may not be repeatable across ontogenetic stages (but see Nespolo and Franco, 2007). Conversely, $\dot{V}_{O_2max}$ measured at the same age in different P_{O_2} was significantly repeatable, suggesting that the physiological basis underlying this trait remains relatively the same in a given time period (despite the testing P_{O_2}).

P_{O_2} effects on aerobic performance

Several studies have compared $\dot{V}_{O_2}$ between high *versus* low altitudes employing measurements at ambient P_{O_2} (e.g. Hammond et al., 2002; Calbet et al., 2003; Chappell et al., 2007). However, this approach does not fully control for P_{O_2} effects during measurements: whereas mice from high altitudes were measured in a hypoxic atmosphere, animals from low altitudes would have been measured in normoxia. Thus, it remains unclear how phenotypic

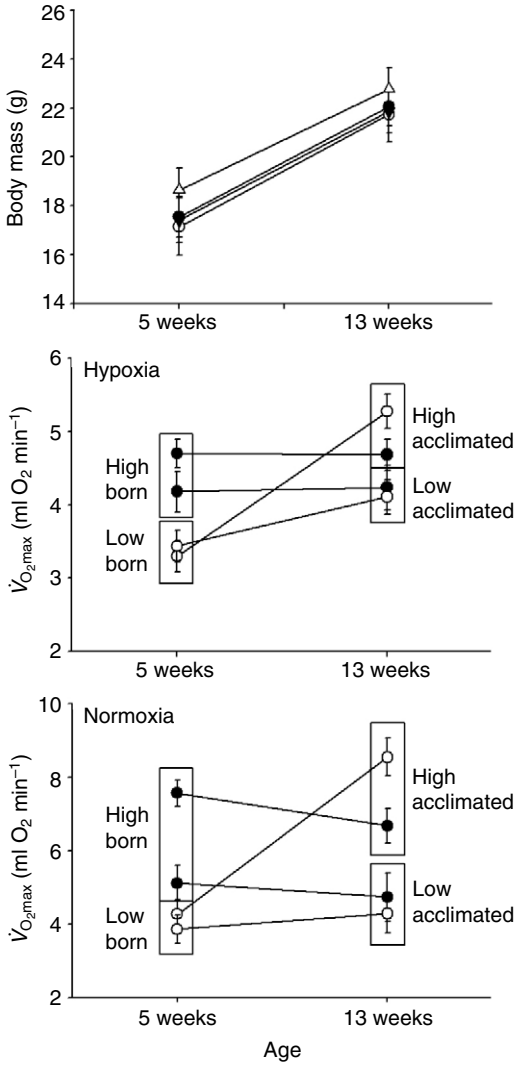

Fig. 4. Top panel, changes in body mass and aerobic performance, measured in hypoxia (middle panel) and normoxia (bottom panel) during the 8 week period between the first and second measurements. Open symbols represent low-born animals and filled symbols represent high-born animals. Means ± s.e.m. are adjusted estimates controlling for within-individual effects.

(anatomical and physiological) responses to chronic exposure to different P_{O_2} affected aerobic performance, because acute effects of ambient P_{O_2} were not accounted for. Our results suggest, for instance, that not accounting for P_{O_2} may underestimate the degree of physiological accommodation following high altitude acclimation. Whereas differences in aerobic performance between groups controlling for P_{O_2} ranged between 21% and 73%, comparisons between high altitude mice measured in hypoxia *versus* low altitude mice measured in normoxia resulted in differences of around 14% (see middle two bars in Fig. 2 and Fig. 3 top panel). As such, most studies in humans (Gonzalez et al., 1998; Calbet et al., 2003; Ventura et al., 2003) and in small mammals like deer mice (Hammond et al., 2002; Chappell et al., 2007) report significantly higher aerobic performance in high P_{O_2} environments than in low P_{O_2} environments. These results, however, might not reflect the acclimatory physiological responses to chronic exposure to different P_{O_2}. This study controls for differences in P_{O_2} across different altitudes by testing animals in multiple P_{O_2} environments, and using this approach we were able to focus on the physiological accommodations made at high altitude and how they affect aerobic performance.

Developmental effects *versus* reversible plasticity during adulthood

We have shown that deer mice residing at 3800 m have a higher $\dot{V}_{O_2max}$, both early (Fig. 2) and later in life (Fig. 3). However, in terms of aerobic performance, does development at high altitude manifest itself differently from acclimation as a low-born adult? Apparently, it does. Our results from regular ANCOVAs and repeated-measures ANOVA show that aerobic performance during adulthood is partly determined by site of development and birth. In this context, main effects of birth site were not significant, suggesting that being born at high or low altitude *per se* does not determine whether mice will have a high or a low $\dot{V}_{O_2max}$ during adulthood. However, the significant interaction term between birth site and acclimation highlights that the outcome of acclimation to different altitudes depends on where mice were born and raised (Figs 3 and 4).

These results suggest that developmental canalization partly accounts for aerobic performance during adulthood, but additional studies are necessary to disentangle which factors underlie the patterns described here. Mice born at low altitude apparently have a larger flexibility to increase $\dot{V}_{O_2max}$ when acclimated to high altitude (Fig. 4), which was quite unexpected and apparently counterintuitive. This result demonstrates that high P_{O_2} during *in utero* development and growth might ultimately enable animals born at low altitude to attain an increased $\dot{V}_{O_2max}$ compared with highlander natives following acclimation to high altitude. In this context, it is worth emphasizing that responses associated with developmental canalization are not necessarily beneficial or adaptive in a Darwinian sense (see Wilson and Franklin, 2002). Instead, they might reflect constraints associated with growing in a more restrictive environment, as might be the case at higher altitudes with lower P_{O_2}. Interestingly, mice born at high altitude cannot decrease $\dot{V}_{O_2max}$ following low altitude acclimation to levels comparable with those of animals born at low altitude. This result reflects 'developmental canalization' in a more traditional sense (Wilson and Franklin, 2002), suggesting that development at high altitude leads to a constrained degree of plasticity in aerobic performance during adulthood.

To our knowledge, this is the first study to report significant developmental effects on aerobic performance during adulthood.

Future studies with similar experimental designs may help in elucidating the physiological basis underlying our results. Aerobic performance is a complex trait that depends on a variety of subordinate traits in the O_2 cascade, hence it is possible that developmental effects may be detected in subordinate organs as we have reported here for whole-individual $\dot{V}_{O_2max}$. Additional research should primarily focus on traits that are known to be affected by acclimation at different altitudes. For example, Hammond et al. (Hammond et al., 1999; Hammond et al., 2001) reported that both heart and lung mass were ~17% higher in deer mice born at and acclimated to high altitude when compared with deer mice maintained at low altitude. Additionally, after acclimation to 3800 m, deer mice increased hematocrit by ~9% (Hammond et al., 1999; Hammond et al., 2001) (G.A.R. and K.A.H., unpublished data). All else being equal, mice with larger cardiopulmonary organs and higher hematocrits should have a higher aerobic performance (Bishop, 1997; Rezende et al., 2006). Thus, it would be worth addressing how subordinate traits at different levels in the O_2 cascade might be affected by different environmental conditions during development (Burggren and Crossley, 2002). For instance, it is possible that mice can develop larger hearts when developing in normoxia, and this might ultimately explain why mice from low altitude attained the highest $\dot{V}_{O_2max}$ following acclimation to high altitude.

Summary and perspectives

Deer mice perform better in normoxic P_{O_2} than they do in hypoxic P_{O_2}, which is consistent with previous results in this species. Here, we show this trend is consistent, regardless of the altitude at which mice reside. This result is important because it illustrates that previous studies, which have cited decreased aerobic performance at high altitude, might be reporting the confounding effects of decreased P_{O_2}, not the real changes in the functional machinery that ultimately determines individual aerobic performance.

Our data also suggest that mice that have undergone gestational development at high altitude might have experienced early, rapid growth of the organs and organ systems that contribute to aerobic performance, and this was manifested functionally as a high aerobic performance at 5 weeks of age. Low-born mice acclimated to high altitude late in life were able to generate a high aerobic performance, especially in normoxia. High-born mice did not experience an increase in aerobic performance in response to 8 additional weeks of exposure to high altitude. In this context, although acclimation during adulthood was able to partly compensate for differences attained during development (see also Chappell et al., 2007), we have detected significant and apparently irreversible effects associated with *in utero* development and growth at a given altitude. Therefore, differences between populations inhabiting high and low altitudes can now be attributed to at least three sources of variation: genetic variation, phenotypic plasticity during adulthood and developmental effects. Future studies should therefore address which subordinate traits in the O_2 cascade are more susceptible to canalization during development, which traits are 'hard-wired' and not open for modification by the environment (Spicer and Gaston, 1999), and how physiological heterokairy of different subordinate traits might ultimately translate into differences in aerobic performance during adulthood.

M. A. Chappell assisted with open flow respirometry set-up, and C. Miller offered logistical assistance. S. Red, A. L. Sport and P. Addison provided husbandry in our absence from the White Mountain Research Station. Finally, we thank E. Hice and J. Urrutia in the UCR Biology machine shop for constructing the treadmill. All animal work was approved by the UCR Animal Care and Use Committee. This work was funded by the National Science Foundation grant no. IBN0073229 to K.A.H. and M. A. Chappell. G.A.R. was supported by a minigrant from the University of California White Mountain Research Station.

REFERENCES

Almeida-Val, V. M. F., Buck, L. T. and Hochachka, P. W. (1994). Substrate and acute temperature effects of turtle heart and liver mitochondria. *Am. J. Physiol.* **266**, R858-R862.

Bartholomew, G. A., Vleck, D. and Vleck, C. M. (1981). Instantaneous measurements of oxygen consumption during pre-flight warm-up and post-flight cooling in sphingid and saturnid moths. *J. Exp. Biol.* **90**, 17-32.

Bishop, C. M. (1997). Heart mass and the maximum cardiac output of birds and mammals: implications for estimating the maximum aerobic power input of flying animals. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **352**, 447-456.

Blacker, H. A., Orgeig, S. and Daniels, C. B. (2004). Hypoxic control of the development of the surfactant system in the chicken: evidence for physiological heterokairy. *Am. J. Physiol.* **287**, R403-R410.

Burggren, W. W. and Crossley, D. A., II (2002). Comparative cardiovascular development: improving the conceptual framework. *Comp. Biochem. Physiol.* **132A**, 661-674.

Calbet, J. A. L., Boushel, R., Radegran, G., Sondergaard, H., Wagner, P. D. and Saltin, B. (2003). Why is V_{O_2max} after altitude acclimatization still reduced despite normalization of arterial O_2 content? *Am. J. Physiol.* **284**, R304-R316.

Chappell, M. A. and Snyder, L. R. G. (1984). Biochemical and physiological correlates of deer mouse alpha-chain hemoglobin polymorphisms. *Proc. Natl. Acad. Sci. USA* **81**, 5484-5488.

Chappell, M. A., Hayes, J. P. and Snyder, L. R. G. (1988). Hemoglobin polymorphisms in deer mice (*Peromyscus maniculatus*): physiology of beta-globin variants and alpha-globin recombinants. *Evolution* **42**, 681-688.

Chappell, M. A., Bachman, G. C. and Odell, J. P. (1995). Repeatablility of maximal aerobic performance in Belding's ground squirrels, *Spermopholis beldingi*. *Funct. Ecol.* **9**, 498-504.

Chappell, M. A., Hammond, K. A., Cardullo, R. A., Russell, G. A., Rezende, E. L. and Miller, C. (2007). Deer mouse aerobic performance across altitudes: effects of developmental history and temperature acclimation. *Physiol. Biochem. Zool.* **80**, 652-662.

Curran, L. S., Zhuang, J., Droma, T. and Moore, L. G. (1998). Superior exercise performance in lifelong Tibetan residents of 4,400 m compared with Tibetan residents of 3,658 m. *Am. J. Phys. Anthropol.* **105**, 21-31.

Dzialowski, E. M., von Plettenberg, D., Elmonoufy, N. A. and Burggren, W. W. (2002). Chronic hypoxia alters the physiological and morphological trajectories of developing chicken embryos. *Comp. Biochem. Physiol.* **131A**, 713-724.

Gonzalez, N. C., Clancy, R. L., Moue, Y. and Richalet, J. P. (1998). Increasing maximal heart rate increases maximal O2 uptake in rats acclimatized to simulated altitude. *J. Appl. Physiol.* **84**, 164-168.

Guderley, H. and St-Pierre, J. (1996). Phenotypic plasticity and evolutionary adaptations of mitochondria to temperature. In *Animals and Temperature: Phenotypic and Evolutionary Adaptation* (ed. I. A. Johnston and A. F. Bennett), pp. 127-152. Cambridge: Cambridge University Press.

Hammond, K. A., Roth, J., Janes, D. N. and Dohm, M. R. (1999). Morphological and physiological responses to altitude in deer mice *Peromyscus maniculatus*. *Physiol. Biochem. Zool.* **72**, 613-622.

Hammond, K. A., Szewczak, J. and Krol, E. (2001). Effects of altitude and temperature on organ phenotypic plasticity along an altitudinal gradient. *J. Exp. Biol.* **204**, 1991-2000.

Hammond, K. A., Chappell, M. A. and Kristan, D. M. (2002). Developmental plasticity in aerobic performance in deer mice (*Peromyscus maniculatus*). *Comp. Biochem. Physiol.* **133A**, 213-224.

Hammond, K. A., Chmura, C. A., Russell, G. A. and Ortiz, S. (2004). Genetic and phenotypic responses of small mammals to life at high altitudes. *Integr. Comp. Biol.* **44**, 564.

Hayes, J. P. (1989a). Altitudinal and seasonal effects on aerobic metabolism of deer mice. *J. Comp. Physiol. B* **159**, 453-459.

Hayes, J. P. (1989b). Field and maximal metabolic rates of deer mice (*Peromyscus maniculatus*) at low and high altitudes. *Physiol. Zool.* **62**, 732-744.

Hayes, J. P. and Chappell, M. A. (1990). Individual consistency of maximal oxygen consumption in deer mice. *Funct. Ecol.* **4**, 495-503.

Hayes, J. P. and O'Connor, C. S. (1999). Natural selection on thermogenic capacity of high-altitude deer mice. *Evolution* **53**, 1280-1287.

Hayes, J. P. and Shonkwiler, J. S. (1996). Altitudinal effects on water fluxes of deer mice: a physiological application of structural equation modeling with latent variables. *Physiol. Zool.* **69**, 509-531.

Hochachka, P. W. (1988). Metabolic responses to reduced O_2 availability. In *Hypoxia: The Tolerable Limits* (ed. J. R. Sutton, C. S. Houston and G. Coates), pp. 41-48. Indianapolis: Benchmark Press.

Hochachka, P. W., Stanley, C., Merkt, J. and Sumar-Kalinowski, J. (1982). Metabolic meaning of elevated levels of oxidative enzymes in high altitude adapted animals: an interpretive hypothesis. *Respir. Physiol.* **52**, 303-313.

Hock, R. (1961). Effect of altitude on endurance running of *Peromyscus maniculatus*. *J. Appl. Physiol.* **16**, 435-438.

McClelland, G. B., Hochachka, P. W. and Weber, J.-M. (1998). Carbohydrate utilization during exercise after high-altitude acclimation: a new perspective. *Proc. Natl. Acad. Sci. USA* **95**, 10288-10293.

McClelland, G. B., Hochachka, P. W., Reidy, S. P. and Weber, J.-M. (2001). High altitude acclimation increases the triacylglycerol/fatty acid cycle at rest and during exercise. *Am. J. Physiol.* **281**, E537-E544.

Nespolo, R. F. and Franco, M. (2007). Whole-animal metabolic rate is a repeatable trait: a meta-analysis. *J. Exp. Biol.* **210**, 2000-2005.
Pough, F. H. (1980). The advantages of ectothermy for tetrapods. *Am. Nat.* **115**, 92-112.
Rezende, E. L., Silva-Duran, I., Fernando Novoa, F. and Rosenmann, M. (2001). Does thermal history affect metabolic plasticity?: a study in three *Phyllotis* species along an altitudinal gradient. *J. Therm. Biol.* **26**, 103-108.
Rezende, E. L., Gomes, F. R., Ghalambor, C. K., Russell, G. A. and Chappell, M. A. (2005). An evolutionary frame of work to study physiological adaptation to high altitudes. *Rev. Chil. Hist. Nat.* **78**, 323-336.
Rezende, E. L., Gomes, F. R., Malisch, J. L., Chappell, M. A. and Garland, T., Jr (2006). Maximal oxygen consumption in relation to subordinate traits in lines of house mice selectively bred for high voluntary wheel running. *J. Appl. Physiol.* **101**, 477-485.
Sheafor, B. A. (2003). Metabolic enzyme activities across an altitudinal gradient: an examination of pikas (genus *Ochotona*). *J. Exp. Biol.* **206**, 1241-1249.
Singer, D. (1999). Neonatal tolerance to hypoxia: a comparative-physiological approach. *Comp. Biochem. Physiol.* **123A**, 221-234.
Snyder, L. R. G. (1981). Deer mouse hemoglobins: is there genetic adaptation to high altitude? *BioScience* **31**, 299-304.
Snyder, L. R. G., Hayes, J. P. and Chappell, M. A. (1988). Alpha-chain hemoglobin polymorphisms are correlated with altitude in the deer mouse, *Peromyscus maniculatus*. *Evolution* **42**, 689-697.
Spicer, J. I. and Burggren, W. W. (2003). Development of physiological regulatory systems: altering the timing of crucial events. *Zoology* **106**, 91-99.
Spicer, J. I. and Gaston, K. J. (1999). *Physiological Diversity and its Ecological Implications*. Oxford: Blackwell Sciences.
Tay, K. L. and Garside, E. T. (1975). Some embryogenic responses of mummichog, *Fundulus heterclitus* (L.) (Cyprinodontidae), to continuous incubation in various combinations of temperature and salinity. *Can. J. Zool.* **53**, 920-933.
Ventura, N., Hoppeler, H., Seiler, R., Binggeli, A., Mullis, P. and Vogt, M. (2003). The response of trained athletes to six weeks of endurance training in hypoxia or normoxia. *Int. J. Sports Med.* **24**, 166-172.
West, J. B. (1991). Acclimatization and adaptation: organ to cell. In *Response and Adaptation to Hypoxia: Organ to Organelle* (ed. S. Lahiri, N. S. Cherniack and R. S. Fitzgerald), pp. 177-190. Oxford: Oxford University Press.
Wilson, R. S. and Franklin, C. E. (2002). Testing the beneficial acclimation hypothesis. *Trends Ecol. Evol.* **17**, 66-70.